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An Allergen-Free Antirabies Vaccine

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Studies on the development of encephalitogenic activity in the cerebral tissue of various animals (rabbits, rats, mice and sheep) showed that the brains of albino rats did not become encephalitogenic until after the 18th day of life, which is later than in any of the other animals studied. On the basis of this finding, a method was developed for the preparation of an entirely allergen-free, non-encephalitogenic antirabies vaccine using the brains of suckling rats. The phenolized vaccine, both in liquid and in lyophilized form, consistently gave high antigenic titres when tested in animals and produced a good increase in virus-neutralizing antibodies in man. It also showed a low thromboplastic activity. More than 1500 litres of this vaccine have since been manufactured on an industrial scale and more than 9500 persons vaccinated. General reactions have been far less frequent than with the conventional Fermi vaccine and no neuroparalytic accidents or shock reactions have been reported. Vaccination with the allergen-free vaccine has proceeded smoothly even in persons considered to be especially at risk owing to previous vaccination with antirabies vaccine or a history of trauma or disease of the central nervous system.

GENERAL CONSIDERATIONS ON THE DEVELOPMENT OF AN ALLERGEN-FREE VACCINE

Vaccines containing brain tissue of adult animals, such as antirabies and anti-tick-borne encephalitis vaccines, are liable to cause allergic post-vaccination accidents of the meningo-encephalomyelitis or polyradiculoneuritis type. These complications are accompanied by paralyses of all grades of severity up to Landry's syndrome and are often fatal.

The frequency of such accidents for the antirabies vaccines varies from 1 case in 600 vaccinations (Pait & Pearson, 1949) to 1 in 7200 (Sellers, 1948) (see also Appelbaum, 1953). The well-known review by Greenwood (1945-46) indicates for vaccines of the Semple type a frequency of neuroparalytic accidents of 1 in 8517, and for vaccines of the Fermi type a frequency of 1 in 7858. From 1956 to 1961, the frequency of neuroparalytic accidents caused by Fermi vaccine increased considerably (Širvinskaja et al., 1963).

The problem remains urgent in most countries of the world. Drastic increases in the frequency of neuroparalytic complications are being reported,

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sometimes in the form of " outbreaks " caused by individual batches of vaccine.

Intense studies of experimental allergic encephalomyelitis (for reviews see Jervis, 1954; Waksman, 1959; Svet-Moldavskij, 1957a; Svet-Moldavskij & Svet-Moldavskaja, 1962) offer a basis for understanding the causes and mechanisms of neuroparalytic accidents.

In our view, the initiation of neuroparalytic accidents is due to the following three factors:

(*a*) an encephalitogenic collagen-like protein (antigen) present in adult brain;

(*b*) additional enhancing factors;

(*c*) immunological reactivity of vaccinated individuals.

Since the level of the encephalitogenic collagen-like protein in the adult cerebrum of a given species is constant, the increase in the encephalitogenic properties of individual series of vaccines must be due mainly to vaccine contamination with additional enhancing factors.

Attention was drawn by Svet-Moldavskij (1957a) to the fact that the brain-containing vaccines may become contaminated with dead phenol-killed bacteria or with bacterial or helminthic products as a result of poor techniques or the use of animals with latent or active infection (for instance, with *Pasteurella, Encephalitozoon cuniculi,* or helminths) and that such contamination results in a marked increase in the encephalitogenic activity of the vaccines, causing mass " outbreaks " of neuroparalytic accidents. These observations have been completely confirmed in the course of time.

Bacterial or helminthic products behave similarly to Freund's adjuvant, although they are certainly far less effective. " Outbreaks " of neuroparalytic accidents have often been noted in various countries, and the use of conventional antirabies vaccines is invariably dangerous.

The strictest possible selection and control of the animals used for the production of antirabies vaccine, absolute sterility of the manufacturing premises, and rejection of any batch that fails to pass even a single sterility test definitely decrease the incidence of neuroparalytic accidents caused by the usual antirabies Fermi vaccines.

The role of immunological reactivity of vaccinated individuals in the development of allergic encephalomyelitis has recently been confirmed by Reznik (1963): in people who developed neuroparalysis following vaccination with antirabies vaccine a higher titre of rabies-neutralizing antibodies was found than in persons vaccinated without complications.

In any sizable human community there are always present some people with increased immunological reactivity more susceptible to allergic encephalomyelitis.

The only way of completely preventing accidents caused by antirabies vaccination is the use of a non-encephalitogenic (allergen-free) antirabies vaccine. The difficulties involved in the preparation of such a vaccine are due, first of all, to the high resistance of the encephalitogenic substance of the brain to chemicals, to high temperatures and to enzymes (Lumsden, 1949; Olitsky & Tal, 1952; Waksman et al., 1954; Roboz et al., 1958; Kies et al., 1958; Kies et al., 1960; and others). The attempts to liberate the antirabies vaccine from the encephalitogenic substance by differential centrifugation (Hottle & Peers, 1954), by treatment with protamine sulfate (Paterson et al., 1953) or by other methods (Bell et al., 1949) have not gone beyond the laboratory stage.[1]

Embryonated egg vaccines, such as the live one prepared from Johnson's and Koprowski's Flury strain (Koprowski & Cox, 1948), used so successfully for prophylactic vaccination of animals, are not suitable for use in man because of the wide occurrence in chick embryos of the lymphomatosis virus (Burmeister & Waters, 1955; Burmeister, 1957) and because of the pathogenicity for a wide range of mammals of at least some oncogenic avian viruses (Svet-Moldavskij, 1957b, 1958a, 1958b, 1959, 1961; Svet-Moldavskij and Skorikova, 1957, 1960; Svet-Moldavskij and Svet-Moldavskaja, 1963; Zil'ber and Krjukova, 1957a, 1957b, 1958; Zil'ber, 1960; Svoboda & Grozdanovič, 1959, 1960; Svoboda, 1960a, 1960b; Landon et al., 1962; Ahlström & Jonsson, 1962, Ahlström & Forsby, 1962; Ahlström et al., 1963). Duck embryo vaccine probably suffers from the same disadvantage and, moreover, is much less immunogenic than vaccine prepared from brain tissue.

On the other hand, no conclusive evidence has ever been produced that in persons infected with street rabies virus the disease can be prevented by vaccination with non-cerebral antirabies vaccines.

[1] We have made many attempts to produce encephalitogen-free vaccine by means of enzyme treatment. Trypsin-treated fresh cerebral tissue shows increased encephalitogenic activity, probably due to destruction of some inhibitors. Collagenase (commercial and purified) does not destroy encephalitogenic substance effectively, either in homogenates of fresh cord tissue or in defatted cord tissue.

In 1955, Svet-Moldavskij and Svet-Moldavskaja suggested another method of preparing non-encephalitogenic antirabies vaccine. This made use of the observation that there is a gradual increase in the amount of encephalitogenic substance during ontogenesis (Svet-Moldavskij & Svet-Moldavskaja, 1955; Svet-Moldavskij, 1957a; Svet-Moldavskaja & Svet-Moldavskij, 1958a,b, 1959, 1962; Svet-Moldavskij et al., 1960; Svet-Moldavskaja et al., 1961; Svet-Moldavskij & Kriegshaber, 1963). This was suggested by the lack of encephalitogenic action of the brain of newborn rabbits, as demonstrated by Kabat, Wolf & Bezer (1947, 1948) in some of the earliest studies of experimental allergic encephalomyelitis.

At first we proposed that an encephalitogen-free antirabies vaccine could be prepared from the brain of newborn rabbits, which has only a slight encephalogenic action up to the 5th postnatal day. However, further observations showed that the brain of suckling rats is more suitable for this purpose, as it is entirely non-encephalitogenic up to the 18th postnatal day. An antirabies phenolized vaccine prepared from the brain of suckling rats proved quite safe and effective and is at present widely used in the USSR.

A continuation of this study by Tuiševa (1963) led to experiments on the preparation from the brain of suckling rabbits of an allergen-free vaccine inactivated by beta-propiolactone.

According to Svet-Moldavskij & Kriegshaber (1963), the brain of suckling rats and the vaccines prepared from it have a lower thromboplastic activity than the conventional vaccines prepared from adult brain.

Quite independently, the production of an allergen-free antirabies vaccine from the brain of suckling mice was proposed by Chilean workers (Fuenzalida & Palacios, 1955; Fuenzalida et al., 1964).

The experimental development of a method of preparing an allergen-free antirabies vaccine in suckling albino rats, the large-scale production of this vaccine (over 1500 litres), and its use in a mass vaccination programme (over 9500 persons) are described in detail below.

MATERIAL AND METHODS

Production of the allergen-free antirabies vaccine

The method of production consists of the following stages: (*a*) the usual passages of the Pasteur strain of fixed rabies virus in rabbits; (*b*) selection of pregnant albino rats and preparation of sucklings; (*c*) inoculation of suckling rats with fixed rabies virus; (*d*) autopsy of the moribund suckling rats, harvesting of brains and testing for sterility; (*e*) homogenization of the dissected brains; (*f*) inactivation of the homogenized suspension of the virus-containing brains in phenolized physiological saline at 20°-22°C; (*g*) bottling and control of the final, liquid vaccine or bottling, lyophilization and control of the final, freeze-dried vaccine.

For the production of the allergen-free antirabies vaccine passages 3248 and 3249 of the Pasteur strain of fixed rabies virus procured from the L. A. Tarasevič State Control Institute of Medical Biological Preparations are used. Sucklings are obtained from clinically healthy pregnant albino rats procured from farms known to be free from infectious diseases. The pregnant rats are thoroughly examined and isolated 3-4 days prior to parturition in individual small cages or perforated pans and subjected to daily examination. The birth date is marked on the label. When 4-8 days old, suckling rats are infected with fixed rabies virus and placed with females (not necessarily with their mothers). Sucklings more than 8 days old should not be infected. On the 3rd or 4th day after infection, distinct signs of rabies are noted in all animals; they lie on one side, with the extremities stiff and extended. Moribund but still living animals are used for vaccine production. They are sacrificed by severing the cervical vessels or the thoracic cage with scissors. Partial exsanguination is thus effected. The sucklings are immersed completely in a 3% phenol solution and transferred to the first sterile room for removal of the skin. The bodies are then painted with iodine solution and transferred to the second sterile room for harvesting of the brain. The cranial case is dissected under sterile conditions and the brain extracted. Dissection is carried out at the level of the cerebellum, which, like the oblongata and spinal cord, is not used for the production of the vaccine but only for inoculation of a broth. Each brain is placed in a separate test tube with 1% phenol solution, and an adjacent test tube containing broth is inoculated with the corresponding cerebellum.

The test tubes inoculated with cerebellum are put into an incubator at +37°C for 5 days, and those containing brain into a refrigerator (+4°C). After completion of the bacteriological test, sterile brains are transferred from the test tubes to a glass or a bottle and homogenized in physiological saline (pH 7.2) containing 1% chemically pure twice

distilled phenol. For the production of the liquid allergen-free antirabies vaccine a 5% brain suspension is prepared in a phenol-containing physiological saline, and for the lyophilized vaccine a 20% brain suspension is used. It has recently been found that for the preparation of the lyophilized vaccine the brain suspension should be prepared in phenolized distilled water rather than in phenolized physiological saline (Pariž, Mučnik, Karakujumčan, unpublished observations), the dried vaccine being subsequently dissolved in physiological saline. The homogenized brain suspension is filtered through silk cloth or 4 layers of gauze, checked for sterility and inactivated in a thermostat at 20°-22°C (8 days for liquid vaccine, 14 days for dried vaccine). As soon as the sterility has been checked and inactivation completed, the liquid vaccine is distributed into ampoules and subjected to final testing. For the preparation of lyophilized vaccine, the 20% brain suspension with 1% phenol, after inactivation at 20°-22°C, is mixed with an equal quantity of the desiccation medium (1% gelatin and 10% glucose or 15% sucrose) filled into 0.75-ml vacuum ampoules, frozen at −70°C, and dried *in vacuo*.

The final allergen-free vaccine is tested for: sterility; safety; immunogenicity; residual virulence; lack of encephalitogenic properties (freedom from allergen); and thromboplastic activity. The dried allergen-free vaccine is tested in addition for: residual humidity; solubility; and presence of vacuum.

The test for encephalitogenic activity is described below. The immunogenicity test is carried out by Habel's method (Habel, 1954), but for the challenge reaction the conventional fixed rabies virus is used instead of CVS.

Thromboplastic activity is assayed by the modified Kudrjašov and Ulitina method (Kudrjašov, 1960) based on the principle of Quick (1940). The rate of clotting of recalcified rabbit plasma is determined in the presence of the allergen-free vaccine and simultaneously in the presence of the brain of adult albino rats. The ratio of the rate of plasma clotting in the presence of the brain of adult rats to that in the presence of the allergen-free vaccine should not exceed 45:70.

Test for encephalitogenic activity

The encephalitogenic activity of brain tissue from animals of various ages was assayed by the technique described elsewhere by Svet-Moldavskij et al. (1959, 1960). For this purpose a 50% suspension of the brain tissue and an equal quantity of Freund's adjuvant were administered subcutaneously to 10-12 guinea-pigs weighing 500-600 g each, doses of 0.2 ml being injected at 5 points on the abdomen (total dose, 1 ml). The animals were kept under observation for 45 days and a record made of neurological signs, such as pareses, paralyses of the extremities and sphincters, and disorders of coordination. As controls, groups of guinea pigs (usually 14 animals per group) were inoculated with a mixture of the same adjuvant with 50% homogenate of adult rabbit brain.

For the test for encephalitogenic activity, a 5% vaccine was centrifuged to precipitate the brain substance and the precipitate resuspended to give a 50% suspension, which was mixed with an equal quantity of Freund's adjuvant and administered to guinea-pigs as described above. The vaccine was considered allergen-free only if none of the 10 inoculated guinea-pigs showed symptoms of allergic encephalomyelitis. In doubtful cases and when clinical signs were absent, the dead animals were thoroughly autopsied and examined histologically.

Tests for encephalitogenic activity were carried out on all experimental series of the vaccines (15 guinea-pigs per series) and on the first five production lots; at present, tests are made on samples taken from every twentieth production lot.

Trials of the allergen-free antirabies vaccine in man.

Inoculations with the allergen-free antirabies vaccine were carried out taking careful note of local and general reactions in the vaccinated person. Special note was taken of people previously inoculated with antirabies vaccine and of those with a history of trauma or disease of the central nervous system. Special records were made of the reactions of the individuals subjected to the following courses of antirabies vaccination, depending on the severity of the exposure: courses of up to 10 injections of 3 ml each; courses of 15 to 30 injections of 3 ml each; and courses of 10 or more injections, each of 5-6 ml of vaccine, followed by a course of 30-40 injections of 3 ml each with revaccination after 10 and 20 days, combined in some cases with injections of antirabies gamma-globulin.

RESULTS

Development of the encephalitogenic substance in the brain of various animals during ontogenesis

A summary of the data on the development of the encephalitogenic substance in the brain will be found in Table 1.

TABLE 1

DEVELOPMENT OF ENCEPHALITOGENIC SUBSTANCE IN THE BRAINS OF DIFFERENT ANIMAL SPECIES DURING ONTOGENESIS

Animal species	Age of animals (days)	Encephalitogenic activity	
		Number of inoculated guinea-pigs	Number of clinically manifest cases of experimental allergic encephalomyelitis
Rabbits	embryos	60	0
	1	60	0
	1-2	16	1
	2	16	3
	4	16	1
	5	16	4
	360	16	11
Mice	5	12	0
	10	12	0
	12	11	0
	16	11	2
	21	11	7
	42	12	5
Rats	4	10	0
	8	10	0
	10	10	0
	15	10	0
	16	10	0
	18	10	0
	20	10	1
	21	10	3
	56	9	7
Sheep	1	15	8
Guinea-pigs	1	15	11

In mouse brain, the encephalitogenic factor is absent up to and including the 12th day after birth. On the 16th day, mouse brain is already capable of inducing experimental allergic encephalomyelitis in guinea-pigs. The brain of rats does not contain the encephalitogenic factor until after the 18th day.

The rabbit brain becomes encephalitogenic as early as the 2nd day after birth, although its encephalitogenic activity is apparently less than that of adult rabbits. Sheep brain is highly encephalitogenic even on the first day after birth and the same applies to the brain of newborn guinea-pigs.

The spinal cord in all the animals tested is already encephalitogenic towards the end of embryonic development.

Development of thromboplastic activity in the rat cerebrum during ontogenesis

A distinct parallelism will be seen in Table 2 between the development of thromboplastic and encephalitogenic activity in the albino rat brain during ontogenesis. Although the absolute values of thromboplastic activity vary from one experiment to another—in particular, according to the nature of the plasma sample—the thromboplastic activity of the brain of new-born rats is invariably lower than that of adults. Similarly, the non-encephalitogenic brain of embryos and new-born rabbits and mice (not presented in Table 2) exhibits a lower thromboplastic activity than the adult brain. On the other

TABLE 2

THROMBOPLASTIC AND ENCEPHALITOGENIC ACTIVITY OF THE ALBINO-RAT BRAIN DURING ONTOGENESIS

Age of rats (days)	Thromboplastic activity		Encephalitogenic activity [a]
	With oxalated plasma (seconds)	With heparinized plasma (minutes)	
0	90-95	>15	0/10
1	85-90	>15	0/10
3	85-90	>15	0/10
8	90-95	>15	0/10
12	—	>15	0/10
16	65-70	7	0/10
20	55-55	6	1/10
21	55-55	5	3/10
25	45-50	3	NT
56	40-45	2	7/9
Plasma without cerebral thromboplastin	130-140	>15	—

[a] The numerator indicates the number of guinea-pigs with clinical signs of experimental allergic encephalomyelitis, the denominator the number of inoculated animals.

NT = not tested.

TABLE 3
STUDY OF EXPERIMENTAL LOTS OF LIQUID AND LYOPHILIZED ALLERGEN-FREE ANTIRABIES VACCINE

Lot No.	Preparation date	Phenol content (%)	Residual virulence	Index of protection [a]	Encephalitogenic activity	Thromboplastic activity [b]
1	1960 May	1	absent	9 400	absent	NT
2	1960 May	1	absent	9 100	absent	NT
3	1960 May	0.5	200	13 000	absent	NT
4 [c]	1960 December	—	100	> 5 000	NT	40/75
6 [c]	1961 March	—	389	> 4 000	absent	40/76
7	1961 March	1	100	> 2 000	absent	40/85

[a] Habel, 1954.
[b] The numerator indicates the thromboplastic activity (in seconds) of the adult albino-rat cerebrum, the denominator, the thromboplastic activity of the tested vaccine.
[c] Lots Nos. 4 and 6 are lyophilized allergen-free vaccine, the remainder are liquid vaccine.
NT = not tested.

hand, the encephalitogenic spinal cord of the newborn animal shows a high thromboplastic activity.

After being defatted successively with various organic solvents—acetone, benzene, ether, chloroform, methanol and ethanol—bovine spinal cord is devoid of any thromboplastic activity although it remains encephalitogenic. The collagen-like encephalitogenic protein prepared according to Roboz et al. (1958) is likewise devoid of thromboplastic activity. On the other hand, various phospholipids isolated from cerebral tissue or synthesized exhibit thromboplastic activity (Rouser et al., 1958; Rapport, 1956; O'Brien, 1957; Therriault et al., 1958). Although these substances are far from identical—thromboplastin is a lipid while the encephalitogenic substance is a protein—the appearance in the brain of encephalitogenic activity coincides with an appreciable increase in its thromboplastic activity. These substances might possibly be constituents of the same macromolecular myelin structures.

In any event, the strict parallel between encephalitogenic and thromboplastic activity has rendered it possible to use thromboplastic activity as an additional but not obligatory criterion when testing allergen-free vaccine prepared from the brains of suckling rats.

A study of experimental and production lots of the allergen-free antirabies vaccine

Suckling rats 4-8 days old were inoculated intracerebrally with fixed virus and on the 3rd-4th day those showing typical signs of disease of the central nervous system were sacrificed. The titre (50% endpoint) of the fixed virus in the brains of these animals was 10^{-6}-$10^{-7.7}$.

Table 3 summarizes the experimental data relevant to the liquid and lyophylized allergen-free vaccine. It will be seen that these lots of vaccine have fairly high immunogenicity and a very low thromboplastic activity, while encephalitogenic properties are altogether absent. These results encouraged us to undertake mass production of the allergen-free vaccine.

Table 4 illustrates the results of testing the first 27 production lots of non-allergic vaccine that have been widely used for antirabies vaccination in man. The results of the tests for encephalitogenic properties are presented in Table 5.

It will be seen from the data in Tables 4 and 5 that the non-allergic antirabies vaccine is highly immunogenic, non-encephalitogenic and exhibits a low thromboplastic activity. Some lots (Nos. 13 and 15) showed high immunogenicity with zero residual virulence. Up to the present, over 1 500 litres of allergen-free vaccine have been produced for mass vaccination of humans.

Samples taken from various lots after the expiry date (5 months) still showed a high immunogenicity (not presented in the table), with a protective index (Habel test) above 1000.

Use of the allergen-free antirabies vaccine in man

Preliminary tests of the allergen-free antirabies vaccine on volunteers and then in small-scale field

TABLE 4

RESULTS OF TESTING PRODUCTION LOTS OF LIQUID ALLERGEN-FREE ANTIRABIES VACCINE WITH 1% PHENOL

Lot No.	Quantity (litres)	Residual virulence (log LD_{50})	Index of protection [a]	Thrombo-plastic activity [b]
1	2.1	1.0	54 950	40/90
2	1.3	2.56	102 300	40/70
3	1.0	2.49	6 761	35/80
4	4.3	1.82	25 120	45/70
5	3.2	2.24	100 000	35/70
6	4.0	2.37	6 160	35/70
7	4.3	1.63	1 738	35/70
8	2.9	1.49	22 390	45/70
9	8.3	1.67	3 467	45/78
10	5.2	1.66	257 000	45/73
11	10.7	1.49	36 310	45/75
12	9.4	1.67	20 890	45/76
13	7.0	0	6 310	45/75
14	7.2	1.5	5 495	45/71
15	8.9	0	34 670	45/78
18	7.6	2.12	154 900	40/80
19	8.0	3.32	95 500	40/74
20	5.4	3.24	309 000	40/85
21	5.6	0.55	9 772	40/70
22	3.7	0.83	19 050	—
23	3.3	1.55	19 500	—
24	4.5	1.16	81 280	—
25	2.5	1.0	11 480	—
226	5.5	0.87	27 540	—
227	1.9	1.0	24 550	—
228	3.5	3.0	56 230	—
230	1.8	1.0	10 720	—

[a] Habel, 1954.

[b] The numerator indicates the thromboplastic activity (in seconds) of the adult albino-rat brain; the denominator is the thromboplastic activity of the tested vaccine.

trials have revealed a low frequency of reactions and a fairly large increase of the virus-neutralizing antibodies. Subsequently, the vaccine was used for a large-scale vaccination programme. Up to the present, over 9500 people have been vaccinated by the allergen-free antirabies vaccine. No vaccination accidents or shock reactions have been recorded among the vaccinated persons.

TABLE 5

ASSAY OF ENCEPHALITOGENIC ACTIVITY OF PRODUCTION LOTS OF ALLERGEN-FREE VACCINE

Material tested (mixture with Freund's adjuvant)	Encephalitogenic activity [a]
Allergen-free vaccine lot No. 1	0/10
„ „ „ „ No. 2	0/10
„ „ „ „ No. 3	0/10
„ „ „ „ No. 4	0/9
„ „ „ „ No. 5	0/10
Control adult rabbit brain	14/14
Allergen-free vaccine lot No. 6	0/8
Control adult rabbit brain	4/10
Allergen-free vaccine lot No. 19	0/6
Control adult rabbit brain	7/10
Allergen-free vaccine lot No. 245	0/8
„ „ „ „ No. 262	0/9
„ „ „ „ No. 282	0/8
Control adult rabbit brain	8/14

[a] The numerator indicates the number of guinea-pigs with clinical signs of experimental allergic encephalomyelitis, the denominator the number of inoculated animals.

A thorough study of local and general reactions such as headache or fever has been carried out in 1000 men vaccinated with the allergen-free antirabies vaccine in Moscow, Penza, and the Georgian SSR.

Tables 6 and 7 summarize the data pertaining to local and general reactions elicited by vaccination with the non-allergic vaccine in Moscow in 1963. Local reactions presented by infiltrations persisting

TABLE 6

GENERAL REACTIONS TO ALLERGEN-FREE VACCINE IN 1963 AS COMPARED WITH GENERAL REACTIONS TO FERMI VACCINE AT THE MOSCOW MUNICIPAL EPIDEMIOLOGICAL CENTRE IN 1962

Vaccine	No. of vaccinated persons	General reactions [a]	
		No.	%
Allergen-free vaccine	342	9	2.6
Fermi vaccine	348	106	30.5

[a] Headache, rise in temperature or nausea.

TABLE 7

LOCAL REACTIONS DURING THE COURSES OF ALLERGEN-FREE VACCINE INJECTIONS IN THE PASTEUR UNIT OF THE MOSCOW MUNICIPAL EPIDEMIOLOGICAL CENTRE

Number of vaccinated people	No. of 3-ml doses of vaccine administered to each person	Local reaction (infiltration)	
		Lasting more than 3 days	Lasting till end of vaccination
15	2-4	0	0
142	5-14	6	0
44	15-24	0	0
5	25-50	1	0
206	—	7	0

for more than 3 days were recorded in 7 out of 206 individuals (3.4%) while general reactions, as exemplified by headache and a rise in temperature to 37.8°C, were noted in 9 cases out of 342 (2.6%). By contrast, among 348 individuals vaccinated in Moscow in 1962 with the conventional Fermi vaccine, general reactions were noted in more than one fourth of the vaccinated people (30.5%).

Similar data have been obtained in Penza and the Georgian SSR.

A special study was carried out on the use of the allergen-free antirabies vaccine in people most susceptible to neuroparalytic accidents, namely, those previously vaccinated with antirabies vaccine and those recovering from disease or trauma of the central nervous system. In 19 persons previously treated with antirabies vaccine (Table 8) and in 26 persons with a history of trauma or disease of the central nervous system, vaccination proceeded quite smoothly.

Six cases have been recorded in which injections of the allergen-free vaccine were commenced after the onset of a severe general reaction caused by the conventional antirabies Fermi vaccine. It was possible to complete the course of vaccination with the allergen-free vaccine without any accidents or side reactions.

It will also be noted that in people with severe serum disease elicited by antirabies gamma-globulin or antitetanic serum, vaccination with the allergen-free vaccine proceeded quite smoothly and the course of the serum diseases was not aggravated by the vaccine. Among 50 persons bitten by definitely rabid animals and vaccinated with allergen-free vaccine, not a single case of rabies has been recorded.

TABLE 8

REACTIONS DURING VACCINATION WITH ALLERGEN-FREE VACCINE OF PERSONS PREVIOUSLY VACCINATED WITH FERMI VACCINE AT THE MOSCOW MUNICIPAL EPIDEMIOLOGICAL CENTRE IN 1963

Number of vaccinated persons	Number of 3-ml doses of allergen-free vaccine administered to each person	Local reactions (hyperaemia with or without infiltration)	General reactions
2	2-4	1	0
7	5-9	4	0[a]
6	10-14	1	0
2	15-24	0	0
2	25-50	2	0
19	—	8	0

[a] One person in this group had headache without any other symptoms.

DISCUSSION

It follows from the experimental data on the development of encephalitogenic substance during ontogenesis of the brain in the animals studied (rabbits, albino rats, mice, guinea-pigs, sheep) that the most suitable animal for the production of non-encephalitogenic vaccine is the suckling albino rat (Table 1). In this animal encephalitogenic activity does not appear in the brain until the 17th or 18th postnatal day. When 6-8-day-old sucklings are used for inoculation with the fixed rabies virus, a non-encephalitogenic vaccine can be safely obtained. The use of 6-8-day-old sucklings provides a safety-margin of approximately 10 days prior to the onset of encephalitogenic activity. Freedom from encephalitogenic activity is ensured by the strictest possible test on guinea-pigs, using vaccine that has been concentrated 10 times (up to 50% brain tissue) mixed with Freund's adjuvant, since guinea-pigs appear to be the most susceptible animals to allergic encephalomyelitis (cf. Svet-Moldavskaja & Svet-Moldavskij, 1959). Any of the conventional antirabies vaccines of the Semple, Fermi or Phillips type produced from the brain of adult animals exhibit high encephalitogenic activity when tested in

this way (50%-90% of the guinea-pigs develop encephalomyelitis). These vaccines, at present widely used all over the world, give neuroparalytic accidents in about 0.01%-0.1% of vaccinated people.

A comparison of the encephalitogenic activities of the conventional antirabies vaccines in guinea-pig tests and in vaccinated humans conclusively shows that a preparation devoid of encephalitogenic activity for guinea-pigs when mixed with Freund's adjuvant will never cause neuroparalytic accidents in vaccinated people. Mass vaccinations of humans at present under way confirm this conclusion. So far over 9500 persons have been vaccinated and no neuroparalytic accidents or rabies infections have been recorded.

In contrast to the conventional antirabies vaccines, accidental contamination of the allergen-free vaccine with killed microorganisms or with other "enhancing" factors carries no risk since even the addition of Freund's adjuvant does not make it encephalitogenic. This is particularly important for the production of vaccines dried *in vacuo*.

To stabilize ordinary antirabies vaccine on drying, Nazarov (1957, 1959, 1961) suggested the use of the conventional medium with gelatin. Such a vaccine has been widely used in this country in veterinary practice since 1956. Selimov et al. (1961) and Votjakov et al. (1957, 1958) suggested the use of this vaccine in man. However, this suggestion has had to be rejected as the presence of gelatin increases the viscosity of the vaccine and this might render it more encephalitogenic. Any adjuvants are dangerous in the vaccines prepared from adult brain. Moreover, incomplete solution of the conventional dry vaccines prior to vaccination or the presence of even minute clumps might increase their encephalitogenic activity. On the other hand, for the allergen-free vaccine all these factors are of minor importance.

The addition of gelatin to the vaccine prepared from the brain of the suckling rats does not render it encephalitogenic (cf. Table 5). Hence, the use of dry allergen-free vaccine is quite safe.

The high titre of fixed virus in the brain of suckling rats ensures good immunogenicity of the vaccine after inactivation with phenol (cf. Tables 3 and 4). Allergen-free vaccine of the Fermi type with slight residual virulence and the completely inactivated allergen-free vaccine of the Semple type are both highly immunogenic. Variations in potency of different vaccine lots when assayed by the Habel test depend largely on differences in titre of the fixed virus used for the challenge.

The high antigenicity of the allergen-free vaccine in the Habel test (Tables 3 and 4) is in complete agreement with its high capacity to protect against street rabies virus, as determined experimentally (Maxumov, 1961, unpublished). Furthermore, in people vaccinated with the non-allergic vaccine an appreciable increase of virus-neutralizing antibodies has been noted. According to Karakujumčan (1963), when paired human sera from 41 vaccinated persons were subjected to the standard serum neutralization test, a serum dilution of 1:1280 neutralized 35-50 LD_{50} of fixed virus in 30 out of the 41 cases. In the remaining 11 cases, the sera neutralized this dose of virus in a dilution of 1:80 or 1:160.

In 50 persons bitten by animals definitely known to be infected with rabies, vaccination with the allergen-free antirabies vaccine invariably protected against the disease.

Local reaction to vaccination with the allergen-free antirabies vaccine was somewhat less intense than that to the conventional Fermi vaccine. Infiltration at the site of injection was rather infrequent. Occasionally, reddening as noted without an infiltration or if infiltration did occur it usually disappeared within a very short time (24-48 hours). General reactions (headache, rise in temperature, etc.) occurred in less than 3% of persons inoculated with the allergen-free vaccine, which is much below the frequency with the usual Fermi vaccine (up to 30%).

Of particular importance is the fact that vaccination with the allergen-free vaccine proceeded quite smoothly in people with a history of neurological disease and in those repeatedly vaccinated with antirabies vaccine. Most striking are those cases in which people who had severe reactions to the conventional Fermi vaccine (headaches, rise in temperature, muscle pains, shock reactions, etc.) completed a course of vaccination with the allergen-free vaccine without experiencing any untoward effects. Not a single case of shock reaction has been recorded among the 9500 patients vaccinated with the allergen-free vaccine. Thus, both experimental evidence and mass vaccination demonstrate the advantages of allergen-free vaccine over the conventional Fermi vaccine.

If it be true that the conventional antirabies vaccine increases the frequency of multiple sclerosis, it appears probable that the use of the non-encephalitogenic antirabies vaccine might eliminate this danger as well.

A consideration of particular importance is the thromboplastic activity of the antirabies vaccines and their effect upon blood coagulation in vaccinated humans. The coagulability of the blood increases drastically even after a single injection of the conventional antirabies Fermi vaccine both in rabbits (Petrova et al., 1960) and in man (Torban et al., 1960). Antirabies vaccines of the usual types are thus very dangerous in the elderly. The allergen-free antirabies vaccine, on the other hand, has a very low thromboplastic activity (cf. Tables 3 and 4) which constitutes another advantage over vaccines of the Fermi and Semple type.

Finally, as far as oncogenic factors are concerned, it is beyond doubt that vaccines prepared in non-cultivated animal tissues are much less dangerous than those prepared in tissue cultures, since in non-cultivated animal tissues the viral agents are in a latent state or their level is too low for direct infection. In other words, the use of whole animal tissues is much less dangerous than the use of tissue cultures. The brain is the organ that is best suited to the purpose, as the blood-brain barrier constitutes a physiological protection against extraneous agents.

From the viewpoint of latent viral agents, rabbits and sheep from which the antirabies vaccine is usually prepared are inferior to suckling albino rats. One of the first latent viruses known in virology was virus III of the rabbit. However, little is known as yet about possible rat viruses.

The haemagglutinating virus described by Kilham & Oliver (1959) and Kilham (1961) remains in the rat organism in a latent state. It is activated and attains a definite titre only when grown in tissue culture. It has not been possible to isolate this virus from the brain either of adult rats or of sucklings, even in tissue culture, nor does it multiply in cultures of human tissues (Moore, 1962).

Paired sera of persons vaccinated with the allergen-free vaccine were subjected to the haem-agglutination-inhibition test with 2 haemagglutinating units of the Kilham rat virus (Svet-Moldavskij et al., to be published). In none of 40 sera tested did we find antibodies to this virus. These data additionally exclude the possibility of contamination of the allergen-free vaccine with this virus or with any of its antigenic substances. At all events, inactivation of the vaccines, in the present case by 1% phenol, largely excludes the hypothetical possibility of contamination with latent viruses.

Economically, the allergen-free antirabies vaccine is fairly profitable. The brain of 8-10-day suckling rats weighs 0.5-0.7 g, so that from the brains of sucklings from the litters of one or two pregnant rats as much vaccine is produced as from the brain of one adult rabbit. For the production of 1 litre of a 5% allergen-free antirabies vaccine the litters of 10-20 pregnant rats are used.

The technique of preparation of the allergen-free vaccine is scarcely more complicated than that for the preparation of the conventional Fermi or Semple vaccine. Moreover, the female remains alive and can be used again.

POSTSCRIPT

By February 1965, more than 12 000 persons had been vaccinated with allergen-free antirabies vaccine without any neuroparalytic accidents or shock reactions.

RÉSUMÉ

La vaccination antirabique classique entraîne parfois chez l'homme des complications neuroparalytiques que l'on attribue à la présence d'un facteur encéphalitogène dans le tissu cérébral des animaux adultes employé pour la préparation des vaccins. Les auteurs, en étudiant les dates d'apparition de cette substance dans le cerveau de différentes espèces animales, ont pu démontrer que, chez le jeune rat, le facteur paralysant n'est présent qu'à partir du 18e jour suivant la naissance.

Ils ont tiré parti de cette particularité pour mettre au point un vaccin antirabique non allergisant préparé sur tissu cérébral de rats blancs âgés de quelques jours. Ils décrivent en détail la technique de préparation de ce vaccin dont les premiers lots, sous forme liquide ou lyophilisée, ont été d'abord expérimentés sur l'animal. Doué d'un pouvoir immunogène élevé et d'une activité thromboplastique faible, le vaccin n'a témoigné en revanche d'aucune propriété encéphalitogène. Chez l'homme, après essai sur des volontaires, il a servi à la vaccination antirabique de petits groupes et finalement a permis l'immunisation de plus de 9500 personnes.

Les résultats ont été particulièrement satisfaisants: le pouvoir protecteur du vaccin non allergisant est attesté par le titre élevé des anticorps neutralisants chez les sujets vaccinés; des personnes mordues par des animaux reconnus enragés n'ont présenté aucun trouble pathologique; la vaccination n'a provoqué ni accidents, ni phénomènes de choc et les réactions générales post-

vaccinales ont été beaucoup moins nombreuses que lors de l'emploi du vaccin Fermi (2,6% et 30,5% respectivement). A Moscou, au cours de la vaccination de 206 personnes, on a observé 3,4% de réactions locales aux endroits d'infiltration.

Les réactions post-vaccinales ont été particulièrement suivies chez les sujets prédisposés aux accidents neuroparalytiques par suite de vaccinations antirabiques antérieures, de maladies et de traumatismes du système nerveux central: chez tous, la vaccination par le vaccin non allergisant a pu s'effectuer sans incidents. Dans 6 cas, la préparation a permis de mener à bonne fin le traitement antirabique de sujets chez lesquels le vaccin Fermi provoquait une forte réaction générale.

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